Guidance for Industry

Considerations for Plasmid DNA Vaccines for Infectious Disease Indications

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For questions on the content of this guidance, contact the Office of Vaccines Research and Review at (301) 827-0655.

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Contains Nonbinding Recommendations

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I. INTRODUCTION

This guidance describes our current recommendations concerning preclinical development and testing of DNA vaccines to prevent infectious diseases. This guidance supercedes the 1996 Points to Consider document. In addition, this guidance finalizes the draft guidance of the same title dated February 2005.

For the purposes of this guidance, DNA vaccines are defined as purified plasmid preparations containing one or more DNA sequences capable of inducing and/or promoting an immune response against a pathogen. Typically, these plasmids possess DNA sequences necessary for selection and replication in bacteria. In addition, they contain eukaryotic promoters and enhancers as well as transcription termination/-polyadenylation sequences to promote gene expression in vaccine recipients, and may contain immunomodulatory elements. DNA vaccines are biological products as set forth in section 351 of the Public Health Service Act (PHS ACT) (42 U.S.C. 262) and are regulated by FDA's Center for Biologics Evaluation and Research (CBER). The principal regulations applicable to DNA vaccines are located in Title 21 Code of Federal Regulations (CFR) Parts 210, 211, 312, 600, 601, and 610. Other guidance documents are available from CBER and may contain information that is relevant to DNA vaccines. Some of these documents are listed below and additional guidance documents may be found on the CBER website (http://www.fda.gov/cber/guidelines.htm) or on the website of FDA's Center for Drugs Evaluation and Research (http://www.fda.gov/cder/guidance/index.htm).

Investigational New Drug Applications (INDs) for DNA vaccines designed to prevent or treat infectious diseases should be submitted to CBER's Office of Vaccines Research and Review (OVRR) where primary review responsibility is assigned. Plasmid DNA products intended for non-infectious therapeutic indications are not addressed in this guidance. INDs for these products should be submitted to CBER's Office of Cellular, Tissue and Gene Therapies (OCTGT).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

In December 1996, FDA issued a guidance document, "Points to Consider on Plasmid DNA Vaccines for Preventive Infectious Disease Indications," (1996 Points to Consider document) to assist the developers of DNA vaccines. That document delineated the manufacturing, preclinical, and clinical issues relevant to the development of DNA vaccines, and described potential safety concerns that we, CBER, recommended vaccine developers address prior to the initiation of phase 1 clinical studies. The recommendations involving DNA vaccine manufacture and testing provided in that document were based on our experiences with other types of vaccines and DNA-based products, including gene therapy agents.

In the intervening years, we have permitted the initiation of phase 1 clinical studies of DNA vaccines for a number of infectious disease indications including malaria, hepatitis B, and human immunodeficiency virus (HIV). The initiation of phase 1 clinical studies is predicated on the manufacturers and/or sponsors of vaccine clinical studies documenting the quality and consistency of plasmid manufacture, combined with extensive preclinical safety studies. Considerable preclinical and clinical experience on plasmid DNA vaccines has been accumulated since the issuance of the 1996 Points to Consider document. This experience has been taken into consideration in revising our recommendations concerning preclinical testing of DNA vaccines.

III. MANUFACTURING ISSUES

The following sections describe the manufacturing information that should be provided for new DNA vaccine products for clinical study under an IND (21 CFR Part 312).

A. Product Manufacture

You should describe in the manufacturing summary all components used during manufacture as well as those present in the final product. You should provide detailed descriptions of the plasmid construction, including the source and diagrams of all plasmids used, and all intermediate recombinant DNA cloning procedures. You should provide the DNA sequence of the entire plasmid present in the Master Cell Bank (MCB) along with an annotated sequence identifying all open reading frames including any unexpected open reading frames and/or other sequence elements. During intermediate

steps in the production process, various methods can be used for identity testing, including restriction enzyme mapping and polymerase chain reaction (PCR). However, complete sequencing of the bulk plasmid vaccine is prefered.

You should describe the genotype, source of the bacterial cells, and the procedures to construct master and working cell banks used for production. Specific guidance for the establishment of MCBs and Working Cell Banks (WCBs) is described in CBER's "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993)." You should test both the MCBs and WCBs to ensure that they are free from bacteriophage and other adventitious agent contamination. We recommend that you establish the genetic stability of the plasmid DNA in the WCB.

The description of the manufacturing process should be sufficiently detailed to enable an assessment of the safety of the product. If lots produced for preclinical safety studies are manufactured differently from lots proposed for clinical use, you should clearly document these manufacturing changes.

B. Bulk Plasmid Product Release Testing

If the bulk and final product are the same (i.e., if production runs yield one lot and no further steps in formulation are performed), then testing as described below may be redundant and unnecessary. You should test bulk plasmid products for the properties described below, and you should use standard assay(s) of adequate specificity and sensitivity. It is understood that in the early stages of product development, these assays may utilize a research grade internal standard. You should evaluate assay methods by testing known amounts of reference materials or spiked samples, or by other appropriate measures, and submit to CBER data documenting assay performance. In addition to bulk and final product release testing, we recommend that you also perform in-process testing to ensure manufacturing consistency and product safety. Prior to the initiation of phase 1 clinical studies, you should initiate stability testing as early as possible to support use of the product for the duration of the proposed clinical investigation.

Typically, the bulk release criteria will include tests for visual appearance and plasmid concentration. We recommend that the fraction of plasmid in supercoiled conformation be included in the bulk release criteria, and that you establish a minimum specification for supercoiled plasmid content (preferably >80%). You may select different release criteria and specifications if they provide data to establish that the selected criteria are predictive of immunogenicity or other intended biological activities.

You should evaluate bulk plasmid preparations for the presence of bacterial host cell macromolecules including DNA, RNA, and protein and set preliminary limits for the maximum level of each of these macromolecules (preferably <1%). As product development progresses, the level of host cell material should be further reduced, as technically and logistically feasible. You should perform a test for pyrogenic substances and include the test results with the final lot release documentation. The Limulus

Amebocyte Lysate (LAL) test is a sensitive indicator of the presence of bacterial endotoxins and endotoxin contamination. The amount of endotoxin in plasmid DNA vaccine preparations should not exceed 40 EU/mg plasmid.

You should include a test to establish the identity of the bulk product. For example, agarose gel electrophoresis of plasmid DNA after restriction enzyme digestion is one test that can be used to identify and distinguish individual plasmids. When a single manufacturing facility is used to manufacture more than one DNA vaccine product, the identity test(s) should be capable of uniquely identifying each plasmid produced in the facility.

You should develop a potency assay. During early clinical development, sponsors will have considerable flexibility in the selection of potency assays. This flexibility could include in vitro measures of transfection efficiency that monitor the transcription and/or translation of the encoded gene(s) or in vivo assays of DNA vaccine immunogenicity. We recommend that quantitative potency assays that evaluate relevant biological activity be developed as product development proceeds. Whenever possible, evidence that the selected potency assay correlates with the immunogenicity or protective activity observed in clinical trials should be provided. We recommend that you maintain retention samples of each lot to facilitate comparisons between lots as assay development progresses. You may choose to discuss the selection and implementation of a potency assay with CBER to ensure acceptability of the design.

C. Final Product Release Testing

You should test the final DNA vaccine product for potency, general safety, sterility, purity, quantity, and identity. The test methods and specifications may be the same as those employed for the bulk product release. If the plasmid product is lyophilized we recommend that you perform a test for residual moisture. You should perform a test for the presence of endotoxin on each lot of final product. In addition to final product release testing, you should also perform in-process testing to ensure manufacturing consistency and product safety.

You should establish acceptance criteria and acceptable limits and report the results for each lot of vaccine to be used for clinical studies.

IV. DNA VACCINE MODIFICATIONS

A. Changes to the Insert or Vector

If the DNA sequence of the insert gene and/or backbone vector of a DNA vaccine are changed, we recommend that you consult with CBER to discuss whether the nature and/or magnitude of the change(s) warrant the conduct of additional preclinical studies and/or the submission of a new IND. You should provide to CBER a description of the

changes in manufacturing process and the results from preclinical safety evaluations of the new (modified) DNA vaccine.

B. DNA Sequence Analysis

An issue of product identity of particular relevance to DNA vaccines concerns the degree to which plasmids should be sequenced before the initiation of phase 1 clinical studies. In 1996, we recommended that manufacturers provide (at a minimum) the sequence of the protein-encoding gene insert. Based on evidence that the plasmid backbone may influence vaccine activity, and recognizing that technological advances since 1996 have facilitated DNA sequencing, we recommend that manufacturers provide the complete sequence of the plasmid before initiating phase 1 clinical studies. As noted in Section III.A. of this document, the sequence should be fully annotated, and should identify any unexpected open reading frames and/or other sequence elements. Some DNA vaccines contain a complex mixture of plasmids, with each plasmid carrying a gene encoding a different antigenic protein. You should grow each plasmid separately. We advise that the identity and amount of each plasmid component in the vaccine preparation be determined to ensure lot to lot consistency.

V. PRECLINICAL IMMUNOGENICITY AND SAFETY

A. General Considerations

Preclinical safety evaluation is required for all new vaccines, including DNA vaccines, prior to their use in clinical studies (21 CFR 312.23(a)(8)). The Good Laboratory Practice (GLP) regulations (21 CFR Part 58) apply to the conduct of non-clinical laboratory safety studies that support or are intended to support applications such as INDs and biological license applications. We recommend that you perform preclinical safety studies on every novel DNA vaccine or DNA vaccine/adjuvant combination. We recommend that all preclinical toxicity and biodistribution/persistence studies evaluate the formulation and method of administration proposed for the clinical study. Additional safety evaluations may be necessary when changes are made, for example, to the formulation or route of administration. We recommend that you consult with CBER prior to submission of your IND to discuss the adequacy of your preclinical safety studies and prior human experience to support proceeding with the investigational vaccine product to Phase I clinical trials.

B. Immunogenicity

We recommend that vaccine immunogenicity be assessed in a relevant animal model whenever possible. This may include the evaluation of antigen-specific antibody titers, seroconversion rates, activation of cytokine secreting cells, and/or measures of cell-mediated immune responses. Unless they are also safety studies, pre-clinical immunogencity studies are not subject to the GLP regulations, which do not apply to

basic exploratory studies carried out to determine whether a test article has potential utility (21 CFR 58.3(d)). For DNA vaccines that encode multiple antigens, you should assess the immune response generated against a representative subset of the encoded antigens.

C. Cytokines

For DNA vaccines that contain immunomodulatory genes (such as cytokine-encoding genes), we encourage preclinical studies in animal species responsive to the encoded human cytokine(s) or models using homologous animal gene(s). Such studies should assess whether modulation of cellular or humoral components of the immune system might result in unintended adverse consequences, such as generalized immunosuppression, chronic inflammation, autoimmunity or other immunopathology. We recommend that you consult with CBER concerning the availability and suitability of animal models to conduct such testing.

D. Prime/Boost Strategies

When more than one type of vaccine is used in a sequential immunization protocol, we recommend that you submit information supporting the safety and tolerability of the dose, schedule, and route of administration of each component proposed for use in the heterologous prime-boost regimen. If existing data are deemed adequate to characterize the potential risks of the prime-boost regimen to study participants, additional toxicology studies may not be necessary. We recommend that sponsors consult with CBER for recommendations on the need for additional toxicology information to support the clinical plans.

E. Autoimmunity

Published preclinical studies indicate that DNA vaccination can activate autoreactive B cells to secrete IgG anti-DNA autoantibodies. However, the magnitude and duration of this response appears to be insufficient to cause disease in normal animals or accelerate disease in autoimmune-prone mice. These preclinical studies suggest that systemic autoimmunity is unlikely to result from DNA vaccination. Similarly, the absence of an immune response against cells expressing the vaccine-encoded antigen (including muscle cells and dendritic cells) suggests that an autoimmune response directed against tissues in which such cells reside is unlikely. Yet the possibility persists that DNA vaccines might idiosyncratically cause or worsen organ-specific autoimmunity by encoding antigens (including cryptic antigens) that cross-react with self. Thus, we no longer recommend that preclinical studies be performed to specifically assess whether vaccination causes autoimmune disease, but recommend that the general welfare of animals in preclinical immunogenicity and toxicity studies continue to be carefully monitored.

In cases where an immune response is induced by a transgene product encoding self-antigen (such as a cytokine, chemokine, surface receptor/ligand, or cryptic self-antigen),

we recommend that you examine potential cross-reactivity with the corresponding endogenous protein. If a persistent immune response against an endogenous protein is detected, we recommend that you evaluate potential adverse effects by studying the analogous animal gene in a relevant animal model. We further recommend that you monitor whether an immune response against the self-antigen is elicited during the clinical trial, and carefully evaluate the effect of this response on trial participants.

F. Local Reactogenicity and Systemic Toxicity Studies

Studies designed to assess systemic toxicity and local site reactogenicity may be combined. We recommend that these studies include at least one immunization beyond that planned for clinical use. We recommend that you use the highest dose of vaccine planned for clinical use. An accelerated schedule of vaccine delivery will be considered by FDA. We recommend that the assessments written into the preclinical study protocols include toxicity to potential target organs, including the hematopoietic and immune systems. We recommend that preclinical studies also include clinical pathology assessments (serum chemistry, hematology, and coagulation tests), and histopathology, encompassing both gross and microscopic assessment of tissues. For additional guidance, sponsors are referred to the "WHO Guidelines on Non-clinical Evaluation of Vaccines" for recommendations concerning the choice of animal model and study design for evaluating local reactogenicity and systemic toxicity (www.who.int/entity/biologicals/publications/en/).

For studies of injection site reactogenicity, you should include detailed clinical observations of the injection site(s) following each vaccine administration and histological evaluations of injection-site tissue obtained from biopsies or term necropsy samples. You should evaluate both short-term and persistent toxicity, preferably by studying separate cohorts of animals 2 to 3 days and 2 to 3 weeks after the final vaccination.

G. Biodistribution, Persistence, and Integration Analysis

Plasmid biodistribution, persistence and integration studies were initially recommended to examine whether subjects in DNA vaccine trials were at heightened risk from the long-term expression of the encoded antigen, either at the site of injection or an ectopic site, and/or plasmid integration. Theoretical concerns regarding DNA integration include the risk of tumorigenisis if insertion reduces the activity of a tumor suppressor or increases the activity of an oncogene. In addition, DNA integration may result in chromosomal instability through the induction of chromosomal breaks or rearrangements.

A typical biodistribution/persistence study assesses the presence of plasmid collected from a panel of tissues at multiple time points ranging from a few days to several months post administration. The panel of tissues typically includes the blood, heart, brain, liver, kidney, bone marrow, ovaries/testes, lung, draining lymph nodes, spleen, muscle at the site of administration and subcutis at the injection site. Plasmid levels are typically

evaluated using a quantitative real time polymerase chain reaction assay (Q-PCR) validated for sensitivity, specificity and the absence of inhibitors. We recommend that the sensitivity of this assay be sufficient to quantify <100 copies of plasmid per microgram of host DNA. A claim of "non-persistence" requires that the amount of plasmid at each site falls below this limit of quantification.

Studies examining plasmid biodistribution/persistence indicate that DNA vaccines prepared from a common plasmid vector but encoding different antigens behave similarly. Conventional intramuscular, subcutaneous, intradermal, and particle-mediated delivery of DNA plasmids rarely results in the long-term persistence of vector DNA at ectopic sites. However, tissue at or near the site of administration frequently contains thousands of copies of plasmid per microgram of host DNA for periods exceeding 60 days. Studies assessing the nature of this DNA indicates that the vast majority is not integrated.

Based on these findings, biodistribution studies may be waived for DNA vaccines produced by inserting a novel gene into a plasmid vector previously documented to have an acceptable biodistribution/integration profile. Biodistribution studies will still be necessary for DNA vaccines utilizing novel vectors, formulations, methods of delivery, routes of administration, or any other modifications expected to significantly impact cellular uptake and/or biodistribution. Before conducting biodistribution/persistence studies, we recommend that you contact FDA for advice concerning the need for these studies.

Based on published studies analyzing the frequency with which DNA plasmids persist and integrate, FDA believes that integration studies are warranted only when plasmid persists in any tissue of any animal at levels exceeding 30,000 copies per ug of host DNA by study termination. If the persistence of DNA plasmid exceeds this threshold, sponsors should evaluate whether the DNA has integrated into the genome of the vaccinated animals. A typical integration study will assess all tissue(s) containing persisting DNA plasmid. We recommend that at least four independent DNA samples be analyzed. Each sample may include DNA pooled from several different donors. Q-PCR is generally used to detect and quantify the amount of plasmid DNA present in each genomic DNA preparation. Unintegrated plasmid DNA may be separated from high molecular weight genomic DNA by gel purification. Concatamer may be eliminated by restriction endonuclease digestion targeting a rare motif present in the DNA plasmid. Specifically designed PCR primers may be used to confirm integration and identify genomic integration sites.

VI. REFERENCES: REGULATIONS AND APPLICABLE GUIDANCE DOCUMENTS, AND RELEVANT PUBLICATIONS

- **U.S. CODE OF FEDERAL REGULATIONS**

21 CFR PART 50 – Protection of Human Subjects
21 CFR PART 56 – Institutional Review Boards
21 CFR PART 58 – Good Laboratory Practice for Nonclinical Laboratory Studies
21 CFR PART 210 – Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs; General
21 CFR PART 211 – Current Good Manufacturing Practice for Finished Pharmaceuticals
21 CFR PART 312 – Investigational New Drug Application
21 CFR PART 600 – Biological Products: General
21 CFR PART 601 – Licensing
21 CFR PART 610 – General Biological Products Standards

- **POINTS TO CONSIDER DOCUMENTS**

Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology (4/85).

Supplement to the Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology: Nucleic Acid Characterization and Genetic Stability (4/92).

Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (7/93).

Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (2/97).

- **INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE (ICH) DOCUMENTS**

ICH; Guideline for Industry: Detection of Toxicity to Reproduction for Medicinal Products (9/94).

ICH; Guideline for Industry: Detection of Toxicity to Reproduction for Medicinal Products: Addendum on Toxicity to Male Fertility (4/96).

ICH; Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products (2/04).

- **FDA GUIDANCE DOCUMENTS**

Guideline on Validation of the Limulus Amebocyte Lysate Test As An End Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products and Medical Devices (12/87).

Guideline for the Determination of Test Residual Moisture in Dried Biological Products (1/90).

FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-Derived Products (4/96).

Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy (3/98).

Guidance for Industry: Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product (1/99).

Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice (9/04).

Guidance for industry: Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications (2/06).

Draft Guidance for Industry: INDs – Approaches to Complying with CGMP During Phase 1 (1/06) (This draft guidance when finalized will represent FDA's current thinking on this topic).

- **PUBLICATIONS RELEVANT TO THE ISSUE OF PLASMID DNA BIODISTRIBUTION, PERSISTENCE, AND INTEGRATION ANALYSIS:**

Martin T, Parker SE, Hedstrom R, Le T, Hoffman SL, Norman J, Hobart P, Lew D. Plasmid DNA malaria vaccine: the potential for genomic integration after intramuscular injection. Hum Gene Ther. 1999; 10(5):759-68.

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Parker SE, Monteith D, Horton H, Hof R, Hernandez P, Vilalta A, Hartikka J, Hobart P, Bentley CE, Chang A, Hedstrom R, Rogers WO, Kumar S, Hoffman SL, Norman JA. Safety of a GM-CSF adjuvant-plasmid DNA malaria vaccine. Gene Ther 2001; 8:1011-1023.

Pilling AM, Harman RM, Jones SA, McCormack NA, Lavender D, Haworth R. The assessment of local tolerance, acute toxicity, and DNA biodistribution following particle-mediated delivery of a DNA vaccine to minipigs. Toxicol Pathol. 2002 May-Jun; 30(3): 298-305.

Kim BM, Lee DS, Choi JH, Kim CY, Son M, Suh YS, Baek KH, Park KS, Sung YC, Kim WB. In vivo kinetics and biodistribution of a HIV-1 DNA vaccine after administration in mice. Arch Pharm Res. 2003 Jun; 26(6): 493-8.

Bureau MF, Naimi S, Torero Ibad R, Seguin J, Georger C, Arnould E, Maton L, Blanche F, Delaere P, Scherman D. Intramuscular plasmid DNA electrotransfer: biodistribution and degradation. Biochim Biophys Acta. 2004 Jan 20; 1676(2): 138-48.

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- **PUBLICATIONS RELEVANT TO THE ISSUE OF PLASMID DNA MODIFICATIONS**:

Krieg AM, Wu T, Weeratna R, Efler SM, Love-Homan L, Yan L, Yi AK, Short D, Davis HL. Sequence motifs in adenoviral DNA block immune activation by stimulatory CpG motifs. Proc Natl Acad Sci USA. 1998; 95(21): 12631-6.

Krieg AM, Davis HL. Enhancing vaccines with immune stimulatory CpG DNA. Curr Opin Mol Ther. 2001; 3(1):15-24.